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DEPHY project: Distillery wastewater treatment through anaerobic digestion and phycoremediation—A green industrial approach

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ABSTRACT

Distilleries produce on an average of 15 L of effluent per litre of alcohol which has the characteristics of BOD as 40,000–50,000 ppm and COD as 80,000–100,000 ppm. In industries, the effluent is treated by anaerobic digestion having twin benefits such as degrading the effluent to an extent of BOD 8,000–10,000 ppm, COD 29,000–35,000 ppm while producing biogas having 55% methane. 0.4 to 0.6 kg of methane is produced per kg of BOD reduced by anaerobic digestion. Effluent after anaerobic digestion is subsequently treated by aeration technique. Treatment through aeration requires high energy for treating the effluent to the standards. Now, due to water crisis, number of industries is following either reverse osmosis (RO) or multiple effect evaporation after anaerobic digestion to recycle the water. RO is employed to recover 60% of water as permeate with COD of 100 ppm. Admitting high pollutant load to the RO process, leads to higher pressure drop across the membrane, increasing its operational and maintenance cost. The pollution profile of the reject is more complicated to tackle. In case of multiple effect evaporators, 550 kcal of energy is required for evaporating 1 L of water. The performance of the evaporators will deteriorate faster with time, due to the high influent load and reducing its life cycle. Hence, it necessitates the requirement of an intermediate treatment which would help to reduce the effluent characteristics of biomethanated wash to an appreciable level and would make the further operations less energy intensive. The major reason for high BOD, COD of effluent is due to the presence of colored compounds such as melanoidin. This review aims at degrading possibility of melanoidin using phycoremediation as an intermediate step between anaerobic digestion and aeration. The biomass thus generated by growing microalgae, will be useful for producing by-products.

Contents

Abbreviations: APHA, American Public Health Association; BOD, biological/ biochemical oxygen demand; COD, chemical oxygen demand; NTU, Nephelometric Turbidity Units; TDS, total dissolved solids; TIFAC, Technology Information, Forecasting and Assessment Council; TOC, total organic carbon; TSS, Total Suspended Solids

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1. Introduction

Chemical composition, turbidity, color and temperature of the effluent categories the severity of the effluent and also decides the required treatment technique. Chemical composition can be in the form of organic and/or inorganic in nature. In the case of high organic content (high BOD), anaerobic digestion is an economical and revenue generating treatment adopted in industries i.e. on an average, every kg of BOD can produce 0.4–0.6 kg of methane. Industries such as distilleries (BOD 40,000–50,000 ppm) [1], dairy (BOD–2500 ppm) [1,2], pulp and paper industry (BOD–4000 ppm) [1,3], slaughter house (BOD 3500–4500 ppm) [1,4] and sugar industry (BOD 1250–2000 ppm) [1,5] are using anaerobic digestion for the generation of biogas from its wastewater. Distilleries are one of the industries with high BOD content in the effluent termed as spent wash. The reason for the high BOD in the spent wash is due to the presence of number of organic compounds such as polysaccharides, reduced sugars, lignin, proteins, melanoidin and waxes [6]. The detailed characteristics of spent wash generated from distilleries are given in Table 1.

Distilleries, for which molasses are raw materials, are encouraged to produce alcohol through yeast fermentation. Distilleries today, play a vital role in Indian economy by its variety of products. Ethyl alcohol of 95.5% purity is produced as the main product from the distilleries. This acts as a mother plant to produce further value added chemicals such as acetaldehyde, acetic acid, ethyl acetate and their derived products. In India, there are about 325 distilleries that approximately produce 2.7 billion litres of alcohol annually. Of the total output of alcohol, about 50–52% is utilized for industrial purposes and the rest is used for potable purposes. The demand for ethanol and products from alcohol has been steadily increasing (TIFAC Report, www.tifac.org.in). Accordingly the effluent generation also will increase with time.

Table 1
Characteristics of spent wash (Reference—TDCL, Trichy); parameters analyzed by APHA Protocol, 21st Edition, 2005.

Parameters	Concentration range
Color	Dark brown
Odor	Sugar smell
Temperature	80–90 °C
pH	4–4.6
Conductivity	26–31 mS/cm
Inorganic TDS	17,160–20,460 ppm
TDS	85,000–110,000 ppm
TSS	4,500–7,000 ppm
COD	85,000–110,000 ppm
Acidity	5,200–8,000 ppm
BOD	25,000–35,000 ppm
Sulphate	13,100–13,800 ppm
Ammoniacal nitrogen	800–1,100 ppm
Chlorides	4,500–8,400 ppm
Phenols	3,000–4,000 ppm
Phosphate	1,500–2,200 ppm
Total nitrogen	4,200–4,800 ppm

Note: °C—degree celsius; mS/cm—millisiemens/centimeter; ppm—parts per million.

There are two types of wastewater produced in distillery. One is non-process wastewater which would be comparatively pure and as such can be recycled. Other is the process wastewater consisting of fermenter sludge, spent lees and spent wash. The fermenter sludge contains yeast (*Saccharomyces cerevisiae*) organism which can be diluted (if necessary) and recycled back to the fermentation unit to maintain yeast concentration. Spent lees which are released from the rectified column of distillation may be cooled and recycled. The conventional batch type distilleries produce 15 kL of spent wash for 1 kL of alcohol generation. In the modern continuous type distillery, spent wash is between 10 and 12 kL per kL of alcohol. It could be further minimized if reboiler is used (TIFAC Report, www.tifac.org.in). The distillery process is shown in Fig. 1.

Anaerobic digestion is followed to produce biogas by treating the spent wash. Anaerobic digestion is a process where organic compound in spent wash is digested by the microorganism (Methanogens) to produce biogas (CH_4 60% and CO_2 40% roughly). On an average 1 m^3 of spent wash produces 38–40 m^3 of biogas. Other products of the anaerobic digester are treated spent wash and digested sludge, both are rich in nutrients. The sludge can be used as manure since it contains more nutrient contents. The anaerobically digested spent wash still has the following characteristics (Table 2) which require further treatment. The major challenges of this anaerobically digested spent wash (ADSW) are the removal of color and treatment of inorganic compounds. If another biological treatment could treat the ADSW for color and inorganic compounds it will solve the effluent problem.

Phycoremediation uses microalgae to treat the ADSW. Growth of microalgae is a photosynthetic process which uses sunlight to synthesize the complicated carbon/inorganic molecule present in the effluent. The advantages of phycoremediation are (i) the nutrients are available in the effluent itself (ii) no further water is added for the growth of microalgae (iii) sufficient light energy required for photosynthesis is available in tropical Countries and (iv) the biomass yield, further can be used for production of biogas or biodiesel or fertilizer. Hence, this review focuses the possibilities of coupling the phycoremediation after anaerobic

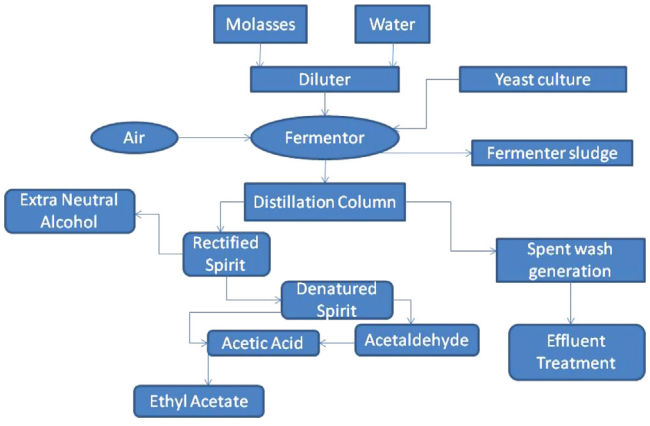

Fig. 1. Distillery plant block diagram—Trichy Distilleries & Chemicals Ltd., (TDCL) Tiruchirappalli.

Table 2
Characteristics of anaerobically digested spent wash (Type—mixed flow reactor)—analysis done at Trichy Distilleries & Chemicals Ltd., Tiruchirappalli.

Parameters	Concentration range
Color	Dark brown
Temperature	35–40 °C
pH	7.5–8.0
Conductivity	31–36 mS/cm
Turbidity (NTU)	40
TDS	35,000–45,000 ppm
TSS	22,000–34,000 ppm
COD	25,000–40,000 ppm
Alkalinity	17,000–24,000 ppm
BOD	7,000–10,000 ppm
Total hardness as $CaCO_3$	3,100–3,200 ppm
Calcium hardness as $CaCO_3$	600 ppm
Magnesium hardness as $MgCO_3$	2,500–2,600 ppm
Ammoniacal Nitrogen as N	1,000–1,100 ppm
Dissolved Phosphate as P	400 ppm
Chlorides as Cl^-	8,400–8,600 ppm
Sulphates as SO_4^-	4,000–4,500 ppm
Fluorides as F^-	5 ppm
Nitrates as NO_3^-	350–400 ppm
Sodium as Na	250–300 ppm
Total Iron as Fe	10 ppm
Oil & Grease	30 ppm
Potassium as K	12,800 ppm
Total silica	60–65 ppm
Reactive silica (Dissolved silica)	50–55 ppm
Bicarbonate	12,800–12,850 ppm

Note: °C—degree celsius; mS/cm—millisiemens/centimeter; ppm—parts per million.

digestion for the complete treatment of spent wash generated from distillery.

2. Mechanism for the formation of complicated color compound—Melanoidin

Spent wash has the characteristic dark brown color. Phenolics (tannic and humic acids) from the feedstock, caramels from overheated sugars, furfural from acid hydrolysis and melanoidins from maillard reaction of sugars (carbohydrates) with proteins (amino groups) mainly contribute to the color of the distillery wastewater [7]. Melanoidins have the antioxidant properties that make them toxic to the aquatic life [8]. Various analytical techniques employed to confirm maillard reaction products were listed by Shen et al. [9], Silvan et al. [10], Jones et al. [11] and Ames et al. [12]. Melanoidins are one of the final products of the maillard reaction. The maillard reaction proceeds at a temperature greater than 50 °C and is favored at pH 4–7 [13]. The pathway for the formation of melanoidins is complex and the structure formed is not fully understood (Fig. 2).

However, Hayase et al. [14] analyzed the functional group present in the model melanoidin. They prepared the melanoidin from glucose and glycine and hydrogen peroxide was used to degrade the melanoidin to analyze the functional groups. According to them, CH–COR moiety and C-terminal structures of melanoidin are originated from glucose. They reported the functional groups existing in the melanodin as CH_3–CO–R, CH–C(H or OH)=C (H or OH)–CO–R″, R–CO–CO–R′, R–CO–CH(CH_3)–CO–R′, R–CO–CH_2–CO–R′, R–CO–CH_2–CH_2–CO–R′, CH_3–CH (OH)–CO–R and so on. With this knowledge, Cammerer et al. [15] prepared various combinations of melanoidin such as glucose and glycine, maltose and glycine, lactose and glycine. They analyzed carbohydrate structures as part of the melanoidin skeleton. Based on the results, for melanoidins formed from carbohydrates and amino acids they suggested a new model of basic melanoidin skeleton (Fig. 3). The outcome of the researches indicate that there is a possibility of

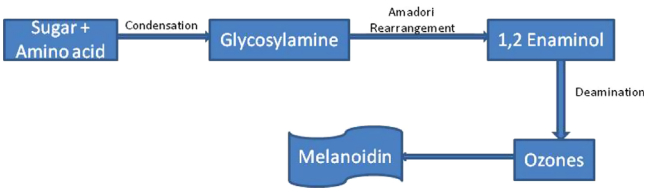

Fig. 2. Hodge's mechanism of melanoidin formation.

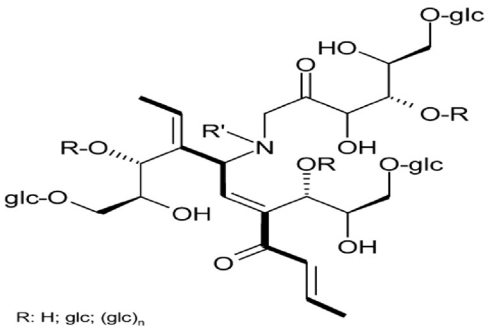

Fig. 3. Structure of basic melanoidin formed from 3-deoxyhexosuloses and ARP [15].

variation in functional groups which are due to the variation in the process temperature, type of carbohydrates (sugars) and type of proteins (Amino acids) present in the melanoidin.

3. Treatment technologies for distillery wastewater

Treatment techniques for distillery wastewater can be categorized as physicochemical treatment and biological treatment techniques. However, there is no individual technique possible which can treat the wastewater completely to the standards. Coupling of physicochemical methods with biological methods is the most promising cascade treatment technology to treat spent wash. Physicochemical treatments have larger limitation due to variation in influent characteristics and they function well at lower pollution load. Biological treatments have larger scope in attacking raw effluent directly. But, a lot of research works are needed to identify right strains or its consortia suitable for the treatment of spent wash, optimization and to improve performance of biological system through molecular/metabolic engineering and simulation techniques.

3.1. Biological treatments

Biological treatments hold good when the BOD/COD ratio is greater than 0.25. The understanding of biochemical reactions exhibited by biological organisms ensure the success of the treatment. Bacterial genera such as *Pseudomonas, Bacillus, Microbacterium, Achromobacter, Staphylococcus, Alcaligens* were used for treating the spent wash [16,17]. Consortia of *Pseudomonas, Stenotrophomonas and Proteus species* were also viable microorganisms to biodegrade and decolorize anaerobically treated spent wash. The treatment results showed that $67 \pm 2\%$ decolorization within 24 h and $51 \pm 2\%$ COD reduction within 72 h when incubated at 37 °C under static condition in biomethanated spent wash supplemented with 0.5% glucose, 0.1% KH_2PO_4, 0.05% KCl and 0.05% $MgSO_4 \cdot 7H_2O$ [18]. Sonal Chaturvedi et al. [19] isolated and characterized rhizosphere bacteria from contaminated site of distillery waste. They observed that 75.5% reduction of color by the same bacteria along with concomitant reduction in COD, BOD, phenol, sulphate and heavy metals values. *Lactobacillus plantarum* No.PV71-1861, showed the potential for decolorization of molasses

wastewater under both anaerobic and facultative conditions. The highest melanoidin pigment removal efficiencies and growth yield of 76.6% and 2.6 mg/mL, respectively were observed within 7 days of culture [20]. *Moringa oleifera* seeds were also used as coagulant for removing color from distillery spent wash [21]. On the whole, distillery spent wash requires the combination of anaerobic biological treatment primarily, followed by aerobic biological treatment to treat it effectively, besides the by-products obtained due to biochemical reaction exhibited by the treating organisms.

4. Anaerobic digestion

This technique is very conventional and still the best in attacking the raw spent wash generated after alcohol separation [22]. Anaerobic biological treatment effectively removes 90% of the COD (70% for the industrial scale), 80–90% of the BOD along with the recovery of 85–90% of biogas generated [23]. Biogas could further be used to generate electricity thereby meeting the power requirement of distilleries. Digestion process is mediated by the action of methanogenic bacteria (Fig. 4; Table 3a and b). Various configurations of anaerobic digesters are in use to reduce the pollution load maximally and/or producing the biogas at the fullest extent [25–30]. The characteristics of biomethanated spent wash after biogas recovery have been shown in Table 2. Calcium and phosphate were found to be detrimental to treatment efficiency [31]. Research is required to reduce the formation of H_2S, removal of calcium and phosphates to improve the treatment efficiency.

4.1. Benefits of anaerobic digestion

Anaerobic digestion process reduces the high pollution load through the production of biogas. Biogas is of high demand now and it majorly fulfills the power requirement of Distillery/Effluent treatment plant (Table 4). Annual bioenergy potential of distillery wastewater for various states of India has been given in Table 5. The production and utilization of biogas also helps to generate

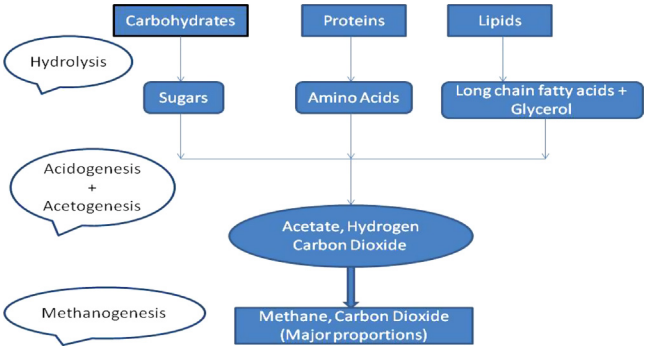

Fig. 4. Anaerobic digestion employed for distillery effluent treatment [24].

Table 3
a and b. Typical biochemical reaction carried out by acidogenic, acetogenic and methanogenic bacteria after hydrolysis in anaerobic digester.

Substrate	Reaction
Lactate	$Lactate + 2H_2O \rightarrow Acetate + CO_2 + H_2O$
Ethanol	$Ethanol + H_2O \rightarrow Acetate + 2H_2$
Butyrate	$Butyrate + 2H_2O \rightarrow 2Acetate + 2H_2$
Propionate	$Propionate + 2H_2O \rightarrow Acetate + 3H_2O_2 + CO_2$
Acetate	$CH_3COOH \rightarrow CH_4 + CO_2$
Hydrogen	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$
Methanol	$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$

Table 4
Biogas generation.

Distillery capacity (kLPD)	Spent wash (kLPD)	Biogas generation Potential (m^3/day)	Energy (kcal)
10	150	6,000	32,040,000
20	300	12,000	64,080,000
30	450	18,000	96,120,000
40	600	24,000	128,160,000
50	750	30,000	160,200,000
60	900	36,000	192,240,000
70	1050	42,000	224,280,000
80	1200	48,000	256,320,000
90	1350	54,000	288,360,000
100	1500	60,000	320,400,000

Note: kLPD—kilo litres per day; m^3—cubic meter; kcal—kilo calories 1 kLPD spent wash produces 40 m^3 of biogas per day. Hence, for a typical distillery capacity of 40 kLPD generates 600 kLPD of spent wash which gives biogas potential of 24,000 m^3 per day. 1 m^3 biogas gives out 5340 kcl energy and thus 24,000 m^3 per day produces 128,160,000 kcl of energy.

Table 5
Annual bioenergy potential of distillery wastewater from various states of India (market characterization report—productive use of methane in Indian distilleries, https://www.globalmethane.org/).

State	Units	Capacity (ML/Yr)	Effluent (ML/Yr)	Biogas (Mm^3)	Biomass (t)
AP	28	143	2,145	58	4,290
Assam	1	2	30	1	60
Bihar	8	54	810	22	1,620
Goa	6	15	225	6	450
Gujarat	10	128	1,920	52	3,840
Karnataka	36	240	3,600	97	7,200
M P	21	469	7,035	190	14,070
Maharashtra	72	692	10,380	280	20,760
Punjab	12	132	1,980	53	3,960
Tamilnadu	22	245	3,675	99	7,350
U P	46	660	9,900	267	19,800
Uttarakhand	4	61	915	25	1,830
W B	6	24	360	10	720
Rajasthan	9	18	270	7	540
Kerala	10	29	435	12	870
Pondicherry	4	15	225	6	450
Sikkim	2	14	210	6	420
Nagaland	1	2	30	1	60
J & K	7	24	360	10	720
H P	3	4	60	2	120
Haryana	8	65	975	26	1,950
Orissa	9	27	405	11	810
Total	325	3063	45,945	1241	91,890

Note: ML/Yr—million litres/year; Mm^3—million cubic meter.

revenue to the industry by means of carbon credits. Carbon credit is obtained due to the generation of power and avoidance of methane entering into the atmosphere. The details are given in Table 6.

4.2. Pre and post physicochemical treatments for anaerobic digestion

To enhance the quality of anaerobically treated spent wash either pretreatment or post treatment is required. Among various techniques being practiced, ultrasound, ozonation and hydrodynamic cavitation are shown to be successful pretreatment techniques to couple with biological treatments for distillery wastewater [32–35]. Ultrasound assisted conventional aerobic oxidation (activated sludge process) resulted in 20% more reduction in COD corresponding to an initial concentration 10,000 ppm [32]. Ultrasound and ozone assisted, thermally and anaerobically treated aerobic oxidation produced 45.6% reduction of COD as compared

Table 6
Power generation and revenue generation by carbon credit (market characterization report—productive use of methane in Indian distilleries).

Distillery capacity (kLPD)	Power generation potential (MW)	Carbon credit due to power generation	Carbon credit due to methane avoidance	Total carbon credit potential per annum	Total carbon revenue (INR)
10	0.38	6	5	3,283	1,969,553
20	0.76	12	20	9,565	5,739,107
30	1.14	18	43	18,248	10,948,660
40	1.52	24	77	30,230	18,138,214
50	1.90	30	120	44,913	26,947,767
60	2.28	36	172	62,296	37,377,321
70	2.65	42	234	82,678	49,606,874
80	3.03	48	305	105,761	63,456,248
90	3.41	54	386	131,843	79,105,981
100	3.79	60	477	160,926	96,555,535

Note: kLPD—kilo litres per day; MW—mega watt; INR—Indian rupees for a typical capacity of 40 kLPD, power generation potential is 1.52 MW, cumulative carbon credit for a day is 101; for an year (appx. 300 days) is 30,230. A carbon credit point earns Rs. 600/- (appx.) therefore for 30,230 points generate total revenue of Rs. 18,138,214/-.

to a mere 1.8% COD reduction for the control [33]. When ozonation technique was applied as a pretreatment technique for distillery wastewater methane yield coefficient and methane production rate were enhanced by around 13.6% and 41.16% respectively [34]. However, reduction of COD was the same as that of common anaerobic digestion, but compounds such as phenols in effluent were converted to other forms and made easier for digestion of spent wash by methanogens [34]. Hydrodynamic cavitation (HC) was evaluated as a pretreatment option for the complex recalcitrant biomethanated distillery wastewater (B-DWW). The HC pretreatment under optimized conditions lead to a biodegradability index of 0.32, COD and TOC reduction of 32.24% and 31.43% respectively along with a color reduction by 48% [35]. Reverse Osmosis (RO) is widely practiced in industries as a post treatment technique to recycle the water for fermentation process use. However, the operational and initial investment costs of RO are high and it possesses the problem of handling the complicated RO rejects. Hence, coupling of economically viable physiochemical treatments with biological treatments will result in the improvement of treatment efficiency.

Several other physicochemical pre and post treatment techniques are inefficient in context to distillery wastewater because of its high pollution profile along with the presence of recalcitrant compound melanoidin. Other reasons include relatively high operational cost and simultaneous generation of secondary pollutants or toxic by-products.

5. Phycoremediation—Post treatment

Microalgae treatment is of superior importance in today's era. It attracts scientist not only by treating waste but also by its products/byproducts which are in high demand. Microalgae treatment is commonly suggested after the anaerobic digestion of spent wash since the process is energy efficient and also microalgae have the ability to take up its nutrients (majorly inorganic compounds) requirement from biomethanated spent wash and energy requirement from the sun. Additionally, it has the mechanism of taking carbon dioxide and converting them into oxygen as electron donor thereby reducing the energy need of the aerobic treatment. The strategy proposed is given in Fig. 5.

Francisca kalavathi et al. [36] considered as pioneer in treating melanoidin present in distillery effluent by the marine cyanobacterium

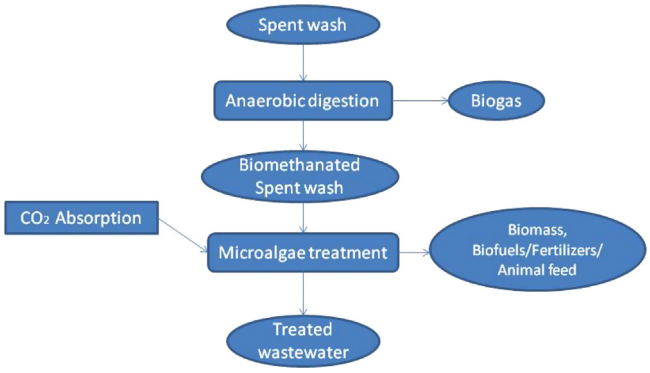

Fig. 5. The strategy proposed for distillery wastewater treatment.

Table 7
Microalgae used for the treatment of various anaerobically digested wastewater [47].

Digested waste	Microalgae species	Reference
Piggery waste	*Arthospira (Spirulina)*	[48]
Swine slurry	*Oocystis sp.* and *Scenedesmus*	[49]
Cow manure (Diluted 1:50)	*Neochloris oleoabundans*	[50]
Poultry waste (Diluted 6%)	*Microalgae consortia*	[51]
Olive mill waste	*Chlorella zofingiensis*	[52]
Food and municipal waste	*Chlorella sorokiniana*	[53]
Municipal wastewater (Secondary effluent)	*Chlorella ellipsoidea*	[54]

Oscillatoria boryana BDU 92181. Afterwards, almost no work is done in India to treat distillery wastewater with focus on decolorization using cyanobacteria/microalgae. However, microalgae treatment has been used for several wastewater treatment for few decades [37–46] (Table 7). Removal of ammonia and phosphate from swine processing wastewater using culture of cyanobacteria (*Chlorella*-like algae) was done in the early days [55]. Immobilization technique was introduced for nutrient removal in wastewater treatment by microalgae (*Chlorella vulgaris*, *Chlorella kessleri* and *Scenedesmus quadricauda*) [56]. Coimmobilization was done between *C. vulgaris* and growth promoting bacterium *Azospirillum brasilense* for the removal of ammonium and phosphorus ions from synthetic and municipal wastewater [57,58]. The effect of starvation on phosphorus removal was studied with synthetic and municipal wastewater source by the microalga *Chlorella sp.* coimmobilized with *A. brasilense* [59]. Heavy metal ions such as Cu (II), Ni(II), Cd(II) and Zn(II) were successfully removed by algal-bacterial consortium [60]. Nitrogen transformations under different conditions in open ponds were studied by Cristina Gonzalez-Fernandez et al. [61] by means of microalgae-bacteria consortium treating pig slurry.

5.1. Microalgae degradation of melanoidin

Melanoidin in the distillery wastewater is the crucial compound which needs attention. Degrading melanoidin after anaerobic digestion is justified since the concentration of organic compound (mainly melanoidin) are quite less than the concentration of inorganic compounds and algae are known for utilizing inorganic compound as nutrients. No literature has given the clear mechanism of melanoidin degradation so far. It is mainly due to the complexity of the maillard reaction and uncertainty about the definite structure of melanoidin polymer. Hence, experiments have been carried out for the chemical and microbial decolourisation/degradation of model as well as natural melanoidin present in sugarcane molasses based distillery wastewaterThe elemental composition and chemical structure of melanoidin polymer mainly

depend on the nature, molar concentration of parent reactants and reaction conditions such as pH, temperature, heating time and water content during the reaction.

Hayase et al. [14] while studying the melanoidin degradation by hydrogen peroxide, investigated the effect of pH on it. They studied the melanoidin decolourisation at different pH (pH 3–pH 13). They found that melanoidin decolourisation in alkaline pH proceeds more rapidly than in acidic and neutral pH and it reached 94% at pH 10. Thus, the degree of decolourisation was found to be pH dependent. Suitable microalgae have to be chosen which can grow in alkaline medium up to the pH of 12 to degrade the melanoidin. According to the report given by Kalavathi et al. [36], enzymes such as glucose oxidase present in microalgae cleave the gigantic melanoidin structure. They also used a cheap enhancer in the form of $MnSO_4$ at an optimal concentration of 175 μM for maximum H_2O_2 production. They further identified enzymes responsible for H_2O_2 production from microalgae that are namely glucose oxidase, Mn dependent peroxidase and at least two Mn independent peroxidases. $MnSO_4$, methylviologen, reduced glutathionine, riboflavin, ascorbic acid could be used for improving the degradation rate of melanoidin by microalgae. Cyanobacteria growing in media containing both pigment and effluent have been demonstrated to possess higher glutamine synthetase activity [36]. Such enzymes help the cyanobacteria in the scavenging of ammonia from amino acid component of the pigment [62]. pH, Temperature, light, oxygen, nutrients and inoculum size are the major environmental factors that largely affect melanoidin degradation [63–65].

Boer et al. [66] had isolated and purified the melanoidin decolourising enzymes (MnP) from *L. edodes*. Two peaks of MnP as MnP1 and MnP2 were obtained by gel filtration. They revealed that the purified enzyme yielded a single band after denaturing SDS-PAGE. A molecular mass of 46 kDa was estimated after SDSPAGE, and this molecular mass was confirmed by Sephadex G-100 gel filtration. *Oscillatoria willei*, when grown under conditions of nitrogen limitation but supplemented with phenolic compound, showed enhanced oxidative stress with a concomitant increase in lignolytic and antioxidative enzyme activities; such as lignin peroxidase, laccase, polyphenol oxidase, superoxide dismutase, catalase, peroxidase and ascorbate peroxidase. It was concluded that these enymes were responsible for the decolorization of substrate phenol up to 52% in 7 days of incubation by the cyanobacterium *O. willei* [67]. The toxic active oxygen species namely, superoxide anion O_2^-, hydroxyl radical OH^- and hydrogen peroxide H_2O_2, are generated in all photosynthetic organisms through electron transport systems [68]. Hydrogen peroxide, a strong oxidizing agent produced by cyanobacteria during the photosynthetic process, is regarded to be fundamentally involved in melanoidin degradation. Evidence for this comes from the fact that in vitro addition of H_2O_2 to melanoidin containing effluent had shown a color reduction of 97% [69].

In another study, *O. boryana* BDU 92181 was found to produce hydrogen peroxide at the rate of 0.538 μmol/μg/Chl/h and was conceptualized to be the major cause of melanoidin degradation [36]. Further, any hydrogen peroxide generated in cyanobacteria during photosynthesis can react with hydroxyl anion to give mainly per-hydroxyl anion [HOO–] which has a strong nucleophilic activity and can also help in decolorisation of melanoidin [70]. Cyanobacteria has also been reported to produce the enzyme superoxide dismutase that leads to the generation of hydroxyl radical and the resultant shift in pH to alkalinity [71,72], which in turn influence the decolorisation and degradation of synthetic melanoidins.

Experiments were conducted to ascertain the melanoidin degradation using microalgae. Melanoidin present in the biomethanated spent wash (2%) [6] is having various amino acids combination since molasses is obtained from sugarcane. Three combinations of Melanoidin were prepared using glucose mixed with glycine (1 M) (G+G), glucose mixed with glutamic acid (1 M) (G+GA) and glucose mixed with both glycine and glutamic acid (1 M) (G+M). The solutions were kept at 121 °C for 3 h in a hot air oven and it was further cooled down to ambient temperature. *Oscillatoria sp.* grown on marine BG-11 media was transferred to the media which contained 100 mL of prepared melanoidin (0.02 M). BG-11 media was replaced and proportion of melanoidin were raised in every subculture in BG-11 media and finally *Oscillatoria sp.* were transferred to media that contained only melanoidin (0.02 M) of G+G, G+GA, G+M combinations. Preliminary melanoidin degradation studies resulted in the increase of conductivity and pH (indirect measurement of microalgae growth) when *Oscillatoria sp.* were used for degradation (Fig. 6a–c). At the end of the batch, microalgae cells were removed by centrifugation and the supernatant was analyzed for total carbon using TOC analyzer (Analytikjena multi N/C 3100). The results indicated that the reduction of total carbon by 4%, 26%, and 24% were observed for G+G, G+GA and G+M samples, respectively. COD and COD absorbance reductions were observed as 2% and 0.5%, 11% and 11%, 20% and 20% for G+G, G+GA and G+M samples respectively (Spetroquant Pharo 300). These observations invoke the elucidation of metabolic pathway that leads to melanoidin degradation and improving the degradation efficiency. The conceptual degradation mechanism has been given in Fig. 7.

5.2. Benefits of growing microalgae for treatment studies

Microalgae are among the most productive organisms on earth. The cellular organism, microalgae also possesses a number of advantages over higher plants as a food source (Fig. 8) and it has a very high potential market value (Table 8). Besides its advantages are CO_2 sequestration and treating the waste through biochemical oxidation.

5.3. Microalgae cultivation on commercial scale

Currently, there are four microalgae cultivation technique available on commercial scale. It includes extensive or open ponds, intensive or raceway ponds, closed photobioreactors in many designs.

5.3.1. Extensive ponds

Large scale extensive ponds for wastewater treatment could be referred from extensive oxidation ponds for sewage treatment—Melbourne Water Corporation's Western Treatment Plant, Werribee, Australia. Cognis Australia Pty Ltd. produces ß-carotene from *D. salina* harvested from hyper saline extensive ponds in Hutt Lagoon and Whyalla. However, these types of systems suffer with the problem of limited mixing and relying on natural site selection [73–77].

5.3.2. Intensive ponds

Intensive ponds are shallow circuits typically of 15–35 cm deep. It has the advantage of cycling the nutrient contents around the pond by the action of paddle wheel. Details could be had from Intensive Pond Production of Spirulina Earthrise Nutraceuticals LLC, California. Microalgae production in these ponds can be as much as 10 times higher than in extensive ponds [73].

5.3.3. Closed photobioreactors

Photobioreactors are closed system and made up of transparent tubes, plates or hemispherical domes. *H. pluvialis* Production in Tubular Photobioreactors (Algatech Corp., Israel) could be referred for this case. It has the advantages such as yield improvement,

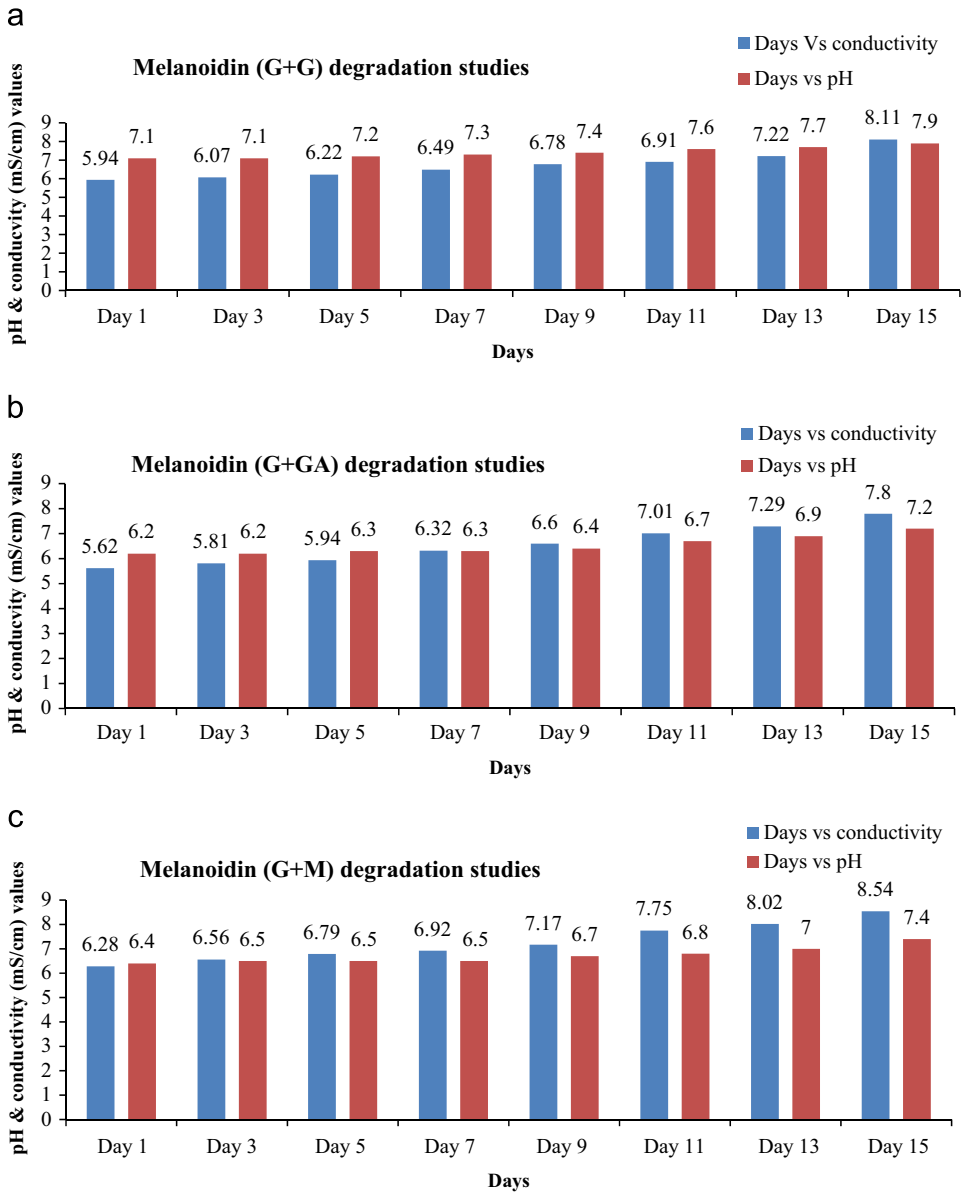

Fig. 6. (a)–(c) Shows increase in pH and conductivity of melanoidin degradation (a) Melanoidin (G+G) degradation (b) Melanoidin (G+GA) degradation (C) Melanoidin (G+M) degradation using *Oscillatoria sp.*

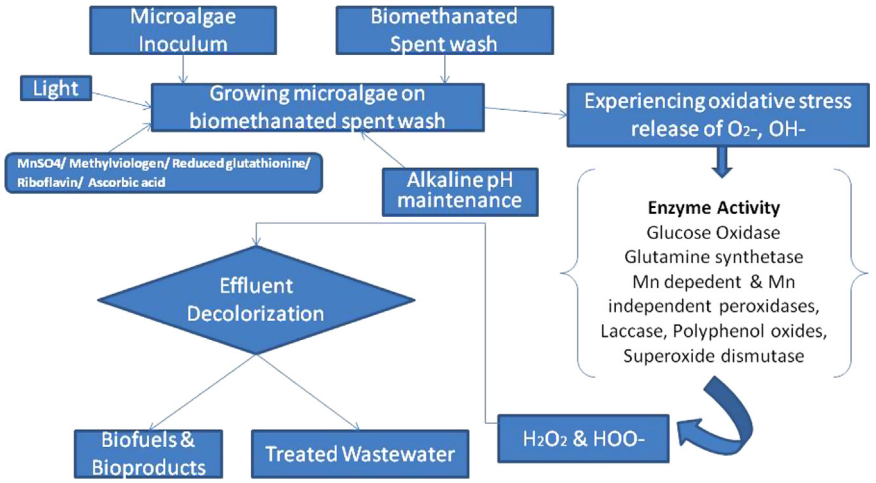

Fig. 7. The conceptual mechanism of melanoidin containing biomethanated spent wash treatment using microalgae.

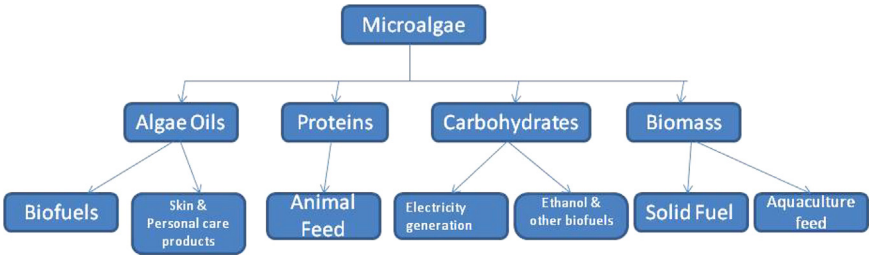

Fig. 8. Microalgae products.

Table 8
Biorefinery of microalgae: bulk chemicals and biofuels in 1000 kg of microalgae (Rene H. Wijjfels, www.algae.wur.nl).

Products	Product value (Euro)	Value (Euro)/tonne of biomass
400 kg of Lipids		
100 kg for Chemical feedstock	2/kg Lipids	200
300 kg Transport fuel	0.5/kg Lipids	150
500 kg of Proteins		
100 kg for Food	5/kg Protein	500
400 kg for Feed	0.75/kg Protein	300
100 kg of Polysaccharides	1/kg Polysaccharides	100
Nitrogen removed-70 kg	2/kg Nitrogen	140
Oxygen produced-1600 kg	0.16/kg Oxygen	256
Total		1646

Note: kg—kilo gram.

Table 9
Comparison of microalgae harvesting methods [82,87,88].

Harvesting operation	Dry solids output conc. (%)
Centrifugation	10–22
Filtration	2–27
Ultrafiltration	1.5–4
Sedimentation	0.5–3
Chemical flocculation	3–8
Flotation	7

Note: conc.—concentration.

avoidance of contamination by pathogens and predators. It also offers benefits that include parameters control and eliminate climate related impacts namely rainfall, evaporation and temperature fluctuations. However, it also has few drawbacks such as fouling due to organisms and mass transfer limitations [74,76].

5.4. Harvesting and biofuel production

Harvesting of microalgae, separation and purification techniques of microalgae products have been well studied by many researchers across the World [78–85]. Harvesting has to be done at an optimum concentration of microalgae cells in the medium to avoid shearing effects and to get an optimum yield continuously. Hence, the microalgae growth process can be operated in a semi continuous mode at large scale [86]. The summary could be seen in the Table 9 and Fig. 9. Latest biotechnological advances have opened door to several alternative routes to produce biofuels (Biodiesel) from microalgae. These breakthroughs include (i) the setup of an hydrothermal liquefaction (HTL) way to convert biomass to bio-crudes directly [89] (ii) the finding that cellular oils can also be secreted into the medium thus bypassing the need to harvest and dry biomass [90] and (iii) microalgae also possess the capacity to synthesize and secrete a range of alkanes/alkenes thus allowing production of drop-in fuel avoiding the expensive chemical processing step (i.e. transesterification) [85].

6. Concluding remarks

Distilleries produce only 7–9% of alcohol (depends on quality) from the cane molasses after fermentation. The rest 91–93% leave as wastewater. The treatment technique should be employed in such a way that the technique/process employed not only reduce the pollution load but also generate some useful byproducts and energy so that it gives dual/triple benefits to the Industry with minimum water foot print. Biological treatments are mostly preferred because this is the only treatment technique that produces very useful byproducts which may be in large demand. Anaerobic digestion of wastewater by methanogens consortia produce biogas and help in meeting power requirements of distillery. Microalgae treatment could be employed after anaerobic digestion because of its potentiality in the wastewater treatment and also its ability to produce diversified products such as biofuels and health care products. However, there are only limited research work carried out in the context of distillery wastewater treatment especially melanoidin degradation.

Microalgae are known to dominate the micro floral populations in many polluted environments and may acquire natural resistance and selectivity against environmental pollutants due to their presence in such polluted systems [91,92]. Native streams of microrganisms are more suitable for the elimination of contaminants such as phenol and phenolic compounds [93]. Consortia can be prepared in synergy with other microalgae/cyanobacteria and need to be tested for its cumulative effort in degradation of compounds that requires detailed research. The distribution of light inside the culture set up is an important factor for microalgae growth hence it is necessary to carry out study on light penetration on the treatment configurations with the help of software tools such as Ansys software. Comprehensive study with focus on the expression of various enzyme complexes in different microalgae at different conditions are required to understand the actual metabolism happening in the microalgae. Several research works are also needed in harvesting and product recovery strategies.

Algae are the only crop capable of replacing the fossil fuel dependency, even though all the potential of microalgae based biorefineries is still in the beginning of its development and many research and development are needed to achieve the desired efficiency and competitiveness. Genetic and metabolic engineering is likely to play an important role in improving microalgae strains to increase the lipid content and the easiness of extraction [94]. Metabolic routes as well as bottlenecks for microbial degradation of pollutants or photosynthetic oxygenation are extensive, which require the need to use a molecular toolbox [95]. Coupling microalgae biomass production with nutrient removal/pollutant degradation may represent an important milestone in the

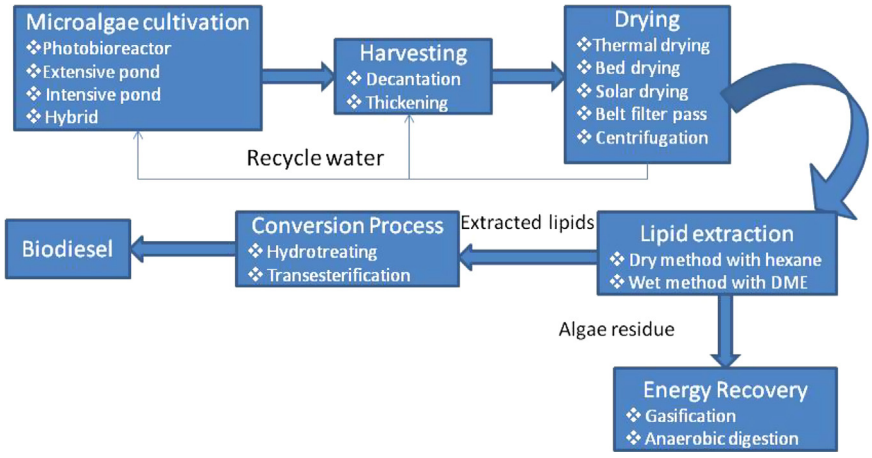

Fig. 9. Microalgae downstream processing steps [85].

bioenergy goals since the wastewater market is immense. However, an appropriate technology for biomass harvesting must be developed to bridge all together.

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